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EXAMINER	
PAK, YONG D	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 11/30/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/031,695	HAUER ET AL	
	Examiner	Art Unit	
	Yong D. Pak	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 September 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-12,14 and 16-18* is/are pending in the application.

4a) Of the above claim(s) *1-11* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *12,14 and 16-18* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

This application is a 371 of PCT/EP00/07252.

The amendment filed on September 19, 2005, amending claims 12 and 16 and canceling claim 15 and adding claim 18, has been entered.

Claims 1-12, 14 and 16-18 are pending. Claims 1-11 are withdrawn. Claims 12, 14 and 16-18 are under consideration.

Response to Arguments

Applicant's amendment and arguments filed on September 19, 2005, have been fully considered and are deemed to be persuasive to overcome the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 12 and claims 16-18 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 12 recites the phrase "isolating resulting hydroxylated product from the medium" and "subterminally hydroxylated aliphatic carboxylic acids". These phrases are not clear to the Examiner. Modified P450 monooxygenases hydroxylate both at the terminal and subterminal positions of a carboxylic acid. Therefore, a) it is not clear to the Examiner how applicants distinguish or direct the enzyme to make only subterminally hydroxylated products and b) it is not clear to the examiner the steps of isolating subterminally hydroxylated carboxylic acids from terminally hydroxylated carboxylic acids. In the context of the above, Examiner takes the position that these claims are incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: steps in isolating subterminally hydroxylated carboxylic acids from terminally hydroxylated carboxylic acids and directing the enzyme towards making only "subterminally hydroxylated aliphatic carboxylic acids".

In response to the previous Office Action, applicants have traversed the above rejection. Applicants argue that it is immaterial whether or not as a by-product terminally hydroxylated products are obtained. Examiner respectfully disagrees. It is material whether or not terminally hydroxylated products are obtained. If they are, it is not clear how subterminally hydroxylated carboxylic acids are separated from terminally hydroxylated carboxylic acids. Further, it is not clear how the enzymes are directed towards making only subterminally hydroxylated aliphatic carboxylic acids.

Applicants also argue that a skilled person may rely on routine methods in order to separate the obtained reaction mixtures. Examiner respectfully disagrees. The

claims do not recite a step of actually isolating "subterminally hydroxylated" products. The claims only recite a step of isolated "hydroxylated products", which includes both subterminally and terminally hydroxylated products. Examiner takes the position that these claims are incomplete for omitting essential steps. Hence the rejection is maintained.

Claim 14 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 14 recites groups of amino acid substitutions, such as in claim 14 part f) "F87A L188K A74G R47F". It is not clear if the amino acid substitutions in these groups is in the alternative or is all-inclusive.

Applicants have not amended the claims to overcome the rejection. Hence the rejection is maintained.

Claim 12 and claims 14 and 16-18 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 12 recites the phrase "is derived from *Bacillus megaterium*". The metes and bounds of this phrase are not clear to the Examiner. Literally, while the term "derived" means "to isolate from or obtain from a source", the above term could also mean "to arrive at by reasoning i.e., to deduce or infer" or also as "to produce or obtain

from another substance". Therefore, it is not clear to the Examiner either from the specification or form the claims as to what applicants mean by the above phrase. It is not clear to the Examiner whether the monooxygnease "derived from *Bacillus megaterium* encompasses a single specific enzyme (SEQ ID NO:2), as in isolated from *Bacillus megaterium,* or whether it encompasses recombinants, variants and mutants of the monooxygenase of SQ ID NO:2 or modified monooxygenase from any other source and labeled as a monooxygenase "derived from *Bacillus megaterium".* As applicants have not provided a definition for the above phrase, Examiner has interpreted the claims broadly to mean that a monooxygenase "derived from *Bacillus megaterium*" encompasses polypeptides which are recombinants, variants or mutants of any monooxygenase. Examiner has given the same interpretation while considering the claims for all other rejections. The rejection can be overcome by amending the phrase to recite "wherein the cytochrome P450 monooxygenase is isolated from *Bacillus megaterium*".

Claims 12 and 14 and claims 16-18 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 12 and 14 recite the phrase "in accordance with SEQ ID NO:2" or "according to SEQ ID NO:2". The metes and bounds of the phrase in the context of the claims are not clear. It is not clear to the Examiner if the recited amino acid sequence has the amino acid sequence of SEQ ID NO:2 or is a representative member of a

genus. Examiner suggests amending the phrase as "the amino acid sequence of SEQ ID NO:2" to clearly indicate that the nucleic acid used in the method encodes the amino acid sequence of SEQ ID NO:2.

Claim 12 and claims 14 and 16-18 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 12 recites the phrase "functional mutation". The metes and bounds of this phrase are not clear to the Examiner. A perusal of the specification did not provide the Examiner with a specific definition for the above phrase. Therefore, it is not clear to the Examiner either from the specification or from the claim as to what applicants mean by the above phrase". Examiner requests clarification of the above phrase.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 12, 14 and 16-18 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 12, 14 and 16-18 are drawn to a method of hydroxylating C8-C12-carboxylic acids or a derivative thereof selected from an alkyl ester, an amide or an anhydride thereof using a enzyme carrying the "mutation F87A" and at least one mutation in the recited regions. However, the limitation that a derivative of C8-C12-carboxylic acids be "selected from an alkyl ester, an amide or an anhydride thereof" and the limitation that if the mutant enzyme comprise F87A, said mutant must comprise another mutation, were not described in the application as originally filed nor in any of its parent applications. Therefore, claims 12, 14 and 16-18 contain new matter.

Given this lack of description of a method of hydroxylating a derivative of C8-C12-carboxylic acids selected from an alkyl ester, an amide or an anhydride thereof, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the inventions of claims 12, 14 and 16-18 at the time of filing of the instant application.

Claims 12, 14 and 16-18 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 12, 14 and 16-18 are drawn to a method for the enzymatic production of subterminally hydroxylated aliphatic carboxylic acids with a cytochrome P450

monooxygenase derived from a *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 having the amino acid sequence of SEQ ID NO:2 comprising at least one mutation recited in the claims or any other amino acid modification and having an altered activity or regioselectivity. Further, the claims are not limited to variants of SEQ ID NO:2 consisting of the recited mutations since the claims recite that the P450 monooxygenases are derived from SEQ ID NO:2. Therefore, the limitation of comprising the recited mutations provides no description on the structure of other parts of the P450 monooxygenase. While the polypeptide used in the method can comprise the recited substituted amino acids, the same polypeptide can comprise any number of amino acids in other positions. Thus, the claims encompass a method for the production of any or all subterminally hydroxylated aliphatic carboxylic acids using any recombinants, variants and mutants of cytochrome P450 monooxygenase derived from a *Bacillus megaterium* cytochrome P450 monooxygenase. Therefore, the claim is drawn to a method of using a genus of polypeptides having any structure to produce a genus of carboxylic acids. The specification only teaches a method for hydroxylating 15-para-nitrophenoxycarboxylic acids (pNCA), 12-pNCA, 10-pNCA or 8-pNCA with a modified cytochrome P450 monooxygenase of SEQ ID NO:2 having mutations at residue 26, 47, 74, 87, 188 or 354 of SEQ ID NO:2 expressed in a host cell comprising a polynucleotide encoding said modified monooxygenases. These limited examples are not enough and does not constitute a representative number of species to describe the whole genus and there is no evidence on the record of the relationship between the structure of a modified cytochrome P450 monooxygenase of SEQ ID NO:2 having

mutations at residue 26, 47, 74, 87, 188 or 354 of SEQ ID NO:2 and the structure of any recombinants, variants and mutants of any cytochrome P450 monooxygenase derived from SEQ ID NO:2. Therefore, the specification fails to describe a representative species of the genus comprising variants and mutants of any recombinants, variants and mutants of any cytochrome P450 monooxygenase derived from SEQ ID NO:2 use to produce the genus comprising any subterminally hydroxylated aliphatic carboxylic acids.

Given this lack of description of the representative species encompassed by the genus of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the inventions of claims 12, 14 and 16-18.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Claims 12, 14 and 16-18 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of enzymatic production of specific subterminally hydroxylated aliphatic carboxylic acids by using a modified cytochrome P450 monooxygenase with SEQ ID NO:2 having single or multiple mutations at residue 26, 47, 74, 87, 188 or 354 of SEQ ID NO:2 with 15-para-nitrophenoxycarboxylic acids (pNCA), 12-pNCA, 10-pNCA or 8-pNCA as substrates,

does not reasonably provide enablement for A) a method for the production of any subterminally hydroxylated aliphatic carboxylic acids and B) wherein said method encompasses the use of any cytochrome P450 monooxygenase derived from SEQ ID NO:2. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims. (See rejection of "derived" under 35 U.S.C. 112, 2nd paragraph).

Factors to be considered in determining whether undue experimentation is required, are summarized in In re Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 12, 14 and 16-18 are drawn to a method for the enzymatic production of subterminally hydroxylated aliphatic carboxylic acids with a cytochrome P450 monooxygenase derived from a *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 having the amino acid sequence of SEQ ID NO:2 comprising at least one mutation recited in the claims or any other amino acid modification and having an altered activity or regioselectivity. Further, the claims are not limited to variants of SEQ ID NO:2 consisting of the recited mutations since the claims recite that the P450 monooxygenases are derived from SEQ ID NO:2. Therefore, the limitation of

comprising the recited mutations provides no description on the structure of other parts of the P450 monooxygenase. While the polypeptide used in the method can comprise the recited substituted amino acids, the same polypeptide can comprise any number of amino acids in other positions. Thus, the claims encompass a method for the production of any or all subterminally hydroxylated aliphatic carboxylic acids using any recombinants, variants and mutants of any P450 monooxygenase derived from SEQ ID NO:2. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of subterminally hydroxylated carboxylic acid and P450 monooxygenase variants and mutants, broadly encompassed by the claims. The claims encompass compounds with widely varying structure and properties. However, in this case the disclosure is limited to a method for hydroxylating 15-para-nitrophenoxycarboxylic acids (pNCA), 12-pNCA, 10-pNCA or 8-pNCA with a modified cytochrome P450 monooxygenase of SEQ ID NO:2 having mutations at residue 26, 47, 74, 87, 188 or 354 of SEQ ID NO:2 expressed in a host cell comprising a polynucleotide encoding said modified monooxygenases. It would require undue experimentation of the skilled artisan to hydroxylate any carboxylic acids.

Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function.

However, in this case the disclosure is limited to a method for hydroxylating 15-para-nitrophenoxycarboxylic acids (pNCA), 12-pNCA, 10-pNCA or 8-pNCA with a modified cytochrome P450 monooxygenase of SEQ ID NO:2 having mutations at residue 26, 47, 74, 87, 188 or 354 of SEQ ID NO:2 expressed in a host cell comprising a polynucleotide encoding said modified monooxygenases. It would require undue experimentation of the skilled artisan to make and use the claimed variants and mutants of any P450 monooxygenases. In view of the great breadth of the claim, amount of experimentation required to make the claimed polynucleotides, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure, the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to use the full scope of the polypeptides encompassed by this claim.

While enzyme isolation techniques, recombinant and mutagenesis techniques are known, and it is routine in the art to screen for multiple substitutions or multiple modifications as encompassed by the instant claims, the specific amino acid positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass a method for the enzymatic production of any or all subterminally

hydroxylating aliphatic carboxylic acids using any or all mutants and variants of any P450 monooxygenase, because the specification does not establish: (A) regions of the substrate binding region of any P450 monooxygenase which may be modified without affecting P450 monooxygenase activity or having an altered substrate profile; (B) the general tolerance of P450 monooxygenase to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residue with an expectation of obtaining the desired biological function; (D) any or all aliphatic carboxylic acids which are subterminally hydroxylated with P450 monooxygenases; (E) a rational and predictable scheme for selecting aliphatic carboxylic acids with an expectation of obtaining a subterminally hydroxylated aliphatic carboxylic acids by incubating said substrates with a modified P450 monooxygenase; and (F) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including a method for the production of any or all subterminally hydroxylated aliphatic carboxylic acids using any or all variants and mutants of any P450 monooxygenase. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of mutants and variants of any P450 monooxygenase having the desired biological characteristics recited in the claim is unpredictable and the experimentation left to those skilled in the art is unnecessarily,

and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

In response to the previous Office Action, applicants have traversed the above rejection.

Applicants argue that the claims have been amended to recite preferred amino acid positions to be mutated and therefore, the claims are not drawn to using any modified cytochrome P450 monooxygenase. Examiner respectfully disagrees. The limitation of comprising substitutions at the recited "preferred amino acids" with an open language such as "at least one amino acid" and the limitation of monooxygenases derived from SEQ ID NO:2 provides no guidance on the structure of other parts of the enzyme. While the polypeptide used in the method comprises can comprise at least one of the recited amino acid mutations, the same polypeptide can comprise any number of amino acids in other positions. Further, the claims are not only limited to mutants of SEQ ID NO:2 but mutants of any cytochrome P450 monooxygenase. Therefore, the claims are drawn to a method of using polypeptides having any structure, including any or all recombinants, mutants and variants, including those that comprise of the recited mutations. As discussed above, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a specific knowledge of and guidance with regard to which specific amino acids in the protein's sequence, can be modified such that the modified polypeptide continues to have said claimed activity. It is this specific guidance that applicants do not provide. Without specific guidance, those skilled in the art will be subjected to undue

experimentation of making and testing each of the enormously large number of mutants that results from such experimentation.

Hence the rejection is maintained.

Claim Rejections - 35 USC § 102

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 12, 14 and 16-18 are rejected under 35 U.S.C. 102(b) as being anticipated by Graham-Lorence et al. (form PTO-1449)

Claims 12, 14 and 16-18 are drawn to a method of producing subterminally hydroxylated aliphatic carboxylic acid by reacting C_8-C_{12}-carboxylic acid derivatives with a modified P450 monooxygenase wherein said modification is a mutation at residue 87 inSEQ ID NO:2 and wherein said modified enzymes shows an altered substrate profile. (See rejection of "derivatives" under 35 U.S.C. 112, 2nd paragraph).

Graham-Lorence et al. discloses a method for producing subterminally hydroxylated aliphatic carboxylic acids with a modified P450 monooxygenase having a valine residue at position 87 of SEQ ID NO:2, wherein the mutant enzyme shows an altered substrate profile (abstract and page 1129). The method of Graham-Lorence et al. uses the reductant recited in claim 17 (page 1127). Therefore, the teachings of Graham-Lorence et al. anticipate claims 12, 14 and 16-18.

In response to the previousOffice Action, applicants have traversed the above rejection. Applicants argue that Graham Lorence et al. does not anticipate the claimed invention because one of ordinary skill will not recognize that arachidonic acid is a

derivative of a C_8-C_{12}-carboxylic acid as defined in the claim. Examiner respectfully disagrees. Because the phrase a derivative of C_8-C_{12}-carboxylic acid "selected from an alkyl ester, an amide or an anhydride thereof" is not clear, an arachidonic acid has been interpreted as a derivative of a C_8-C_{12}-carboxylic acid. Further, absent of any scientific or objective evidence illustrating that arachidonic acid is not a derivative of C_8-C_{12}-carboxylic acid. Therefore the rejection has been maintained.

None of the claims are allowable.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Yong Pak whose telephone number is 571-272-0935. The examiner can normally be reached 6:30 A.M. to 5:00 P.M. Monday through Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization where this application or proceeding is assigned are 571-273-8300 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Yong D. Pak
Patent Examiner 1652

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